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Steroidal Sapogenins. LVIII. Steroidal Sapogenins from the Joshua Tree

The Joshua tree, **Yucca brevifolia,** *is distributed over parts of Arizona, California, Nevada, and Utah. A study of its steroidal sapogenins showed that the leaves contain practically none; the wood, small amounts of smilagenin; the seeds high amounts of hecogenin and tigogenin.*

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The Joshua tree, *Yucca brevifolia* Engelm., forms extensive open forests of dichotomously branched, small trees (Fig. 1) in the region from extreme southwestern Utah southward into Arizona and westward across southern Nevada into southern California. These forests usually occur at altitudes of 3,000 to 5,000 feet.

The number of inflorescences on the trees varies from year to year, but when abundant they form a desert crop grown without human assistance. Cattle and sheep are known to relish the flowers (15). Attempts to find a practical human food use for the flowers reported by Woodbury et al. (15) were not successful because of a soapy flavor of the delicious-looking, otherwise delicately-palatable cooked flowers. The chemical analysis indicated a nutritive content about equivalent to that of many vegetables or fruits.

A search for ways and means of utilizing the huge crop of Joshua tree flowers led to the studies reported herein. The search was further stimulated by the meetings of the International Arid Lands Conference at Albuquerque, New Mexico, in 1955 that emphasized the need to utilize the deserts of the world. We then decided to initiate preliminary studies on the character of the saponins that produced the soapy taste in the flowers. A preliminary sample, taken May 21, 1955 from the Beaver Dam Mountains in Extreme southwestern Utah, indicated a sufficiently high sapogenin content to warrant further investigation.

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The Joshua Tree

The tree yucca was first described and named *Yucca draconis* var. *arborescens* by John Torrey (8) in 1856 from specimens that had been collected on the Pacific Railroad explorations (9). In 1871, while revising data on the genera Yucca and Agave, George Engelmann described and named the same tree *Yucca brevifolia.* It has also been placed in a separate genus, *Clisto-yucca.* The common name, according to tradition, was given by early day Mormons, who asserted the trees with their peculiar shaped arms were, like the Biblical Joshua, pointing the way to the Promised Land.

Joshua trees are well distributed over parts of four states within the area shown on the map (Fig. 2). In many places the trees form open groves or forests. In other places, they may be scattered over the landscape. They are far from continuous within their range, being interrupted in many places by the absence of suitable conditions. They do not grow in the bottoms of the low arid basins nor on the steep slopes of the mountains. They thrive best on the higher gravel slopes that skirt the mountains, usually in definite belts or zones. This belt varies in altitude, usually in conformity with the

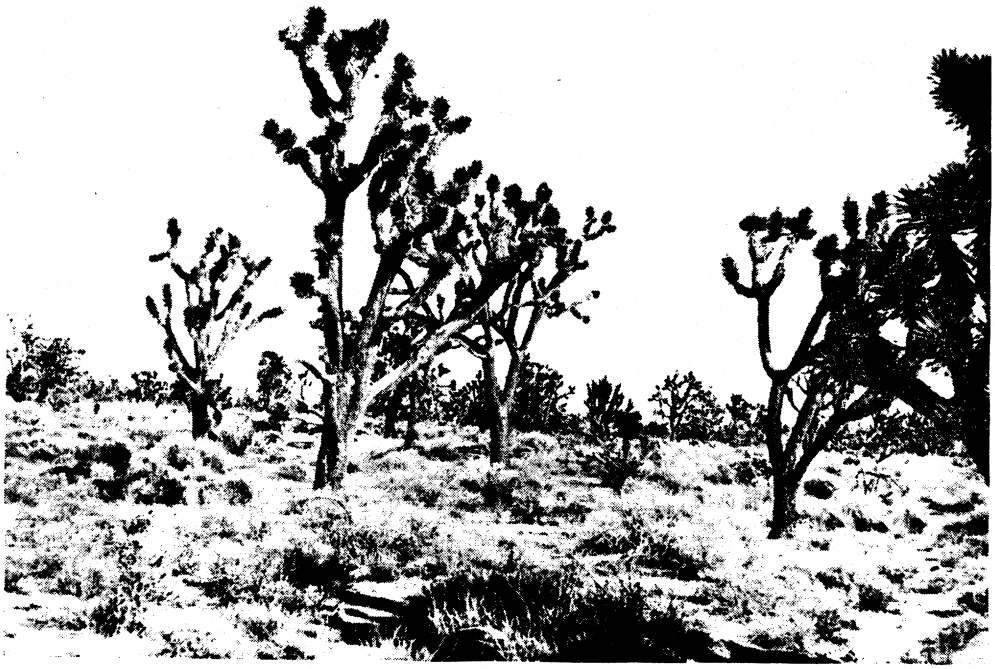


Fig. 1. Joshua tree (*Yucca brevifolia*) forest.

base level from which the slopes rise.

Both observations and measurements indicate that the Joshua is a slow-growing tree. In Joshua Tree National Monument, studies of growth rates conducted over a period of years by members of the Monument staff under the leadership of Chief Ranger H. L. Earenfight, show that the growth rate of seven different trees averaged from 1.36 to 3.48 inches per year. The greatest rates of growth occurred in trees about five to ten feet in height.

The six-chambered capsules, one to two inches in diameter and two to three inches in length may be full of thin black seeds. Each seed is about 0.25 to 0.5 inch in diameter and 0.06 inch in thickness. During development many of these seeds are eaten by insect larvae (presumably yucca moth) and usually only a few mature. Ripening capsules are often eaten by rodents.

The number of inflorescences that develop vary from year to year, from tree to tree, and often from place to place. During favorable years there may be heavy crops; in unfavorable years there may be few. A relatively heavy stand in eastern Nevada about 15 to 20 miles west of Caliente, covering roughly 50,000 acres, was examined on April 17 and 18, 1957. In a favored spot, where the trees were thrifty and relatively dense, a count on 100,000 square feet showed 110 relatively large trees bearing 892 flower panicles. This was a typical heavy crop. In a moderately dense stand a half-mile distant, an area of the same size yielded 95 slightly smaller trees bearing 192 panicles. The flower heads usually weighed between one and two pounds: extra large

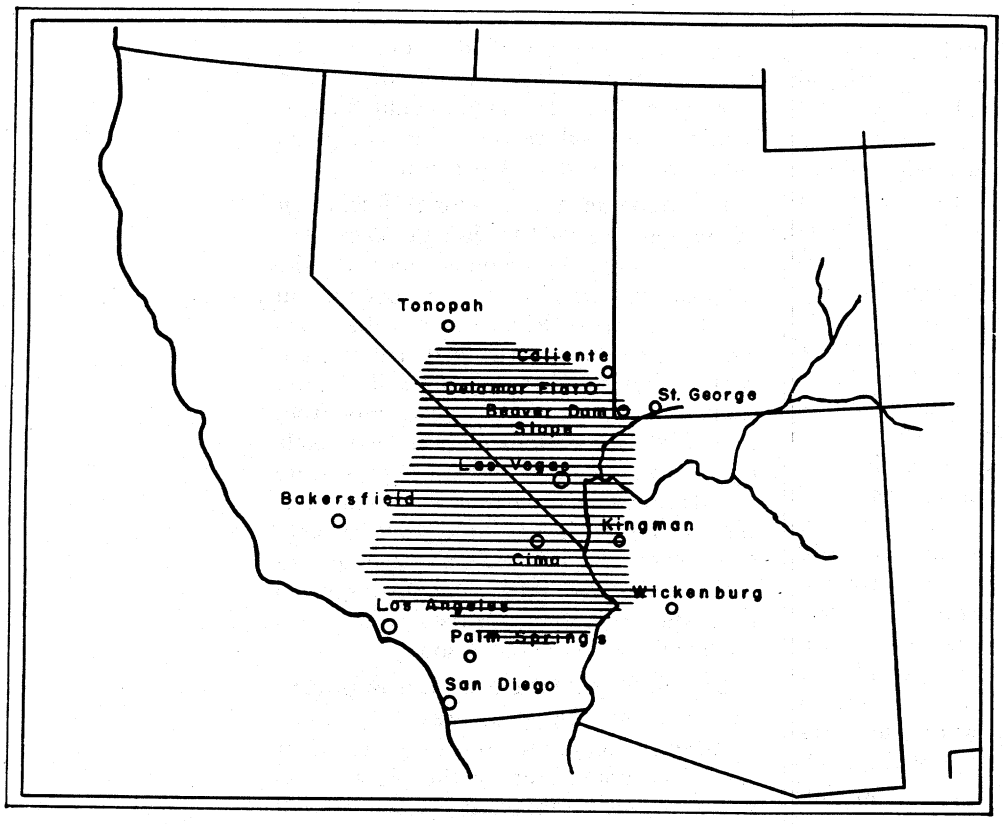


Fig. 2. Map showing important Joshua tree area.

ones reached three pounds. It was roughly estimated that an average of the two stands might contain one tree per 1,000 square feet and bear five panicles of two pounds each, or ten pounds per tree.

A similar stand covering about the same area on the western foothill slopes of the Beaver Dam Mountains in southwestern Utah, examined on April 19, 1957, had passed the fresh blooming period, and was developing large green capsules. A sample area of 10,000 square feet in a medium dense stand yielded a count of 20 trees, 26 inflorescences, and 88 capsules. In a denser stand containing thrifty trees, 20 to 30 capsules per inflorescence were commonly found. However, inflorescences of 40 capsules were occasionally found, and in one case more than 50 occurred.

Steroidal Sapogenins

Analytical Methods. Samples of the various Joshua tree fractions were collected in Utah and Nevada, packed in plastic bags and shipped by air express to the Eastern Utilization Research and Development Division. The material, even the flowers, arrived in excellent condition. The following fractions were made: leaves, which were subdivided into green (younger) and brown (older) fractions; buds, flowers, and their respective stalk fractions; fruits which wherever possible were separated into seeds and empty capsules; and wood. A diagrammatic sketch showing the various plant fractions is shown in Fig. 3.

All the plant fractions were immediately dried in a forced draft hot air oven

at a temperature of 80° C. The dried fractions were then ground to pass a 1 mm. screen. The preferred quantity of sample for sapogenin analysis was 100-200 grams. In many cases quantities as low as 5-10 grams were used with suitable modifications in technique (4). The sample was suspended in 1.5 N HCl, refluxed five hours, and filtered. The insoluble residue containing the sapogenins was washed with bicarbonate solution and water until neutral and then dried at 80° C. The dry residue was placed in a Soxhlet extractor and continuously extracted with heptane for 24 hours. On concentration of the heptane solutions 90-95% of the total sapagenin content could be obtained in crystalline form. The sapogenins were filtered, dried and weighed. Infrared (1, 10) and paper chromatographic analysis (4) showed that only smilagenin, tigogenin, and hecogenin were present. Smilagenin, when present, was always found as the sole constituent, but tigogenin and hecogenin were often present as mixtures. Estimation of the intensity of the infrared carbonyl band gave values for hecogenin which were in reasonable agreement with data obtained by direct isolation of hecogenin by the Girard T reagent (6). Tigogenin could then be obtained by subtracting the hecogenin value from the total sapogenin.

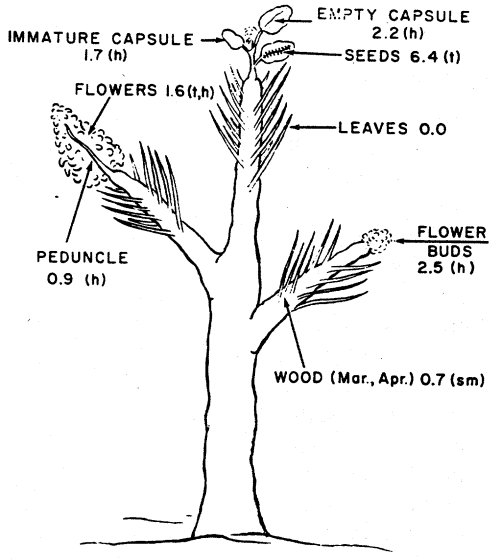


Fig. 3. Diagram showing average sapogenin content in various parts of Joshua tree. (t = tigogenin; h = hecogenin; sm = smilagenin)

Distribution and Seasonal Variation of Sapogenins in the Joshua Tree. Table 1 presents the complete data on the seasonal variation of the sapogenins in the Joshua tree. Fig. 3 shows the average sapogenin content of the various plant parts. On the whole the data collected on samples in two different years and from two different locations were in reasonable agreement. Only three sapogenins were found: smilagenin in the wood and mixtures of tigogenin and hecogenin in varying proportions in the floral parts and in the capsules and seeds. Surprisingly, no sapogenin was found in the leaves. This point will be discussed below.

Chemical Structure of the Sapogenins. The chemical structures of the three sapogenins found are shown in Fig. 4. It should be noted that, in the plant, sapogenins are never found as the free steroid but are combined with sugars through glycosidic linkage at the three position (1, 7) to form the water-soluble saponins. It will be noted that tigogenin and hecogenin are identical except that the latter has a carbonyl group in position 12 of ring C. Hecogenin can be converted easily by chemical means to tigogenin; similarly, enzymes capable of oxygenating C-ring deoxy systems are well known. Hence, it is not surprising that tigogenin and hecogenin should be associated, and, in fact, the association of these two sapogenins is well known (5, 10, 11, 12, 13). Smilagenin has a different A/B ring fusion from that which is found in tigogenin or hecogenin and there is no simple chemical or biological means of converting the *cis* A/B fusion of smilagenin to the *trans* A/B fusion of tigogenin. Marker and his associates, working with the whole plant, found mixtures of A/B

STEROIDAL SAPOGENIN CONTENT OF THE JOSHUA TREE[1]

TABLE I

Date and Location[2]	Wood	Leaf	Inflorescence: Flower Buds: Beginning	Inflorescence: Flower Buds: Developed	Inflorescence: Mature Flowers: Peduncle	Inflorescence: Mature Flowers: Flowers	Fruit: Capsule with Seed	Fruit: Empty Capsule	Fruit: Seed
3/10/58 BD	0.5(sm)	0	2.2(h)						
3/20/56 BD	1.2(sm)	0	0.9(0.4)			2.0(0.4)[3]			
3/26/58 BD	0.1(sm)	0		2.1(0.4)					
	1.2(sm)			1.7(0.3)					
4/ 9/56 BD			3.1(0.3)	2.6(1.0)	1.0(0.4)				
			0.5(h)	4.3(0.4)	1.6(0.4)				
					0.9(2.3)	1.2(1.0)			
					0.4(0.4)	0.8(0.4)			
4/12/58 BD	0.6(sm)	0							
	0.6(sm)								
4/12/58 DF				2.7(0.7)					
4/23/56 BD	0.9(sm)	0			1.1(0.7)	1.6(0.4)	1.0(0.7)[5]		
					1.0(1.0)	4.4(1.0)			
					0.5(2.3)	0.9(2.3)			
4/26/58 DF	0	0		2.4(1.0)	2.1(0.4)				
				2.2(1.0)	1.3(0.5)				
					0.6(h)	1.2(0.7)			
4/26/58 BD	0	0				1.9(1.0)			
5/ 3/57 DF		0				1.7(2.3)			
BD		0					1.0(0.3)		
5/ 7/56 BD				0.9(1.0)[4]	2.2(1.0)[4]			2.6(2.3)	5.0(t)
5/10/58 DF	0	0		0.3(0.2)	1.2(1.0)			2.3(0.6)	8.1(t)
				0.2(0.3)	1.3(1.0)				
5/23/56 BD	0.1(sm)	0		0.9(0.4)[4]	1.6(0.7)[4]		2.5(0.3)	1.9(0.1)	4.5(2.3)
								1.9(0.3)	5.7(2.3)
6/ 1/58 DF	0	0		0.3(h)	1.6(1.0)[4]			2.0(h)[5]	8.0(t)[5]
								1.9(h)	8.9(3.0)
7/19/56 BD	0.1(sm)	0						1.8(2.3)	
8/ 1/58 DF	0				1.9(0.3)[4]				
10/20/58 BD	0.5(sm)	0							
1/ 5/59 BD	0								
4/12/58 DF								1.0(h)[3]	8.7(3.0)[3]

[1]Total sapogenins in percent of dry matter. Figure in parentheses is the ratio, tigogenin/hecogenin; (t) means all tigogenin; (h) means all hecogenin; (sm) means all smilagenin.

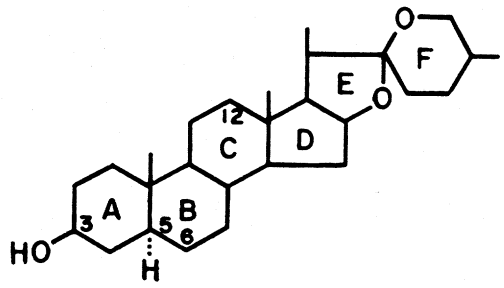
[2]BD means Beaver Dam, Utah; DF means Delamar Flat, Nevada.

[3]Remains from previous year.

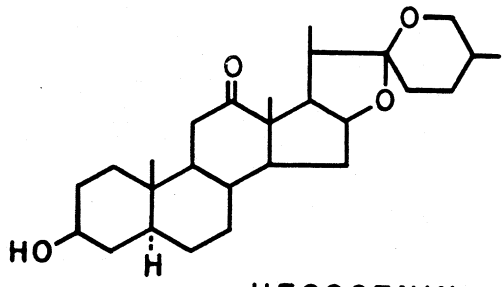
[4]Dry flowers and peduncles.

[5]Immature.

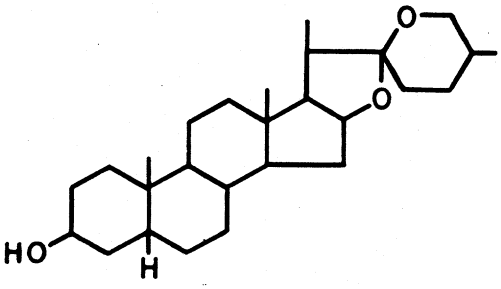
trans and A/B *cis* sapogenins, and on this basis postulated that the two forms are interconvertible (5). In the present study we have been unable to demonstrate the occurrence of the Δ^5-unsaturated precursor. Furthermore, extensive studies cursor. Furthermore, extensive studies of carefully separated plant fractions (10, 11, 12, 13), indicate that in any one tissue A/B *trans* and A/B *cis* sapogenins are never found together. The question of the biogenesis of the two forms remains open.



TIGOGENIN



HECOGENIN



SMILAGENIN

Fig. 4. Structure of tigogenin, hecogenin, and smilagenin.

The data shown in Table 1 and Fig. 3 show several distinct features. Smilagenin is found only in the wood tissue and only during March and April, coincident with the early phases of the reproductive cycle. At no period were significant quantities of sapogenins found in the leaves. Although Table I shows the content as zero, this is not strictly correct. Our analytical procedures are insensitive to sapogenin levels below 0.1-0.2%. In most cases, the leaf samples had quantities of sapogenin between 0.01 and 0.1% which were identified as mixtures of hecogenin and tigogenin. In contrast to our experience with the leaves of many other *Yucca* species (10, 11, 12, 13), saponins are not concentrated or stored to any extent in the leaf of *Yucca brevifolia*.

The largest concentration of saponins takes place in the reproductive parts of the Joshua tree. Flower buds and flowers average between 1.5 and 2.5% total sapogenin as a mixture of hecogenin and tigogenin. On the whole, hecogenin is predominant although the mixture was found in virtually all samples analyzed. A striking increase occurs in the sapogenin content of the fruits. The capsules contain from 2-3%, which is almost exclusively hecogenin. In the seeds the content rises strikingly to values which range between 4.5 and 9.0% with tigogenin now often the exclusive and always the predominant sapogenin. This appears to be a case of biological reduction of the 12-carbonyl group in hecogenin to a methylene moiety in tigogenin.

The large concentration of saponin in the seed of *Y. brevifolia* is not an isolated occurance; a number of *Yucca* and *Agave* species exhibit similar behavior (14). The saponin concentration of Joshua tree seeds, assuming that four sugar moities are combined with the steroid (2), would, in the highest cases, be about 18%, an extraordinarily high value for a substance

with no known metabolic functions. In this connection, a most interesting review by Fraenkel (3) points out that a number of secondary plant substances, including glycosides of steroidal alkaloids which have structures very similar to the steroidal saponins, may serve as general insect or animal repellants and in some cases also as attractants for specific insects. It is of interest that certain moths are invariably found in Joshua tree flowers and their seed capsules.

Utilization of Joshua Tree Sapogenins. Hecogenin is a commercial source of cortisone. Tigogenin can be converted to androgenic and estrogenic hormones. The flow sheet outlines a process by which both sapogenins could be obtained. It should be emphasized that, because of the large development of *Dioscorea* tubers as a source of steroids, bulk steroids such as the above now command a relatively low price. Consequently, the process shown will not be economic unless collection costs are low and large quantities of suitable plant material are available.

FLOW SHEET

FOR

PREPARATION OF HECOGENIN AND TIGOGENIN FROM JOSHUA TREE

Joshua Tree Buds, Flowers or Fruit

1. Macerate tissue
2. Add water and press

Juice	*Bagasse*—dry—possible cattle feed

3. Add sulfuric acid to pH of 1.0
4. Heat near boiling 4-6 hours
5. Cool and decant supernatant liquor from sludge

Sludge (partially hydrolyzed saponin)	Acid liquor (discard)

6. Slurry with 3N sulfuric acid and boil 5 hours
7. Cool, decant liquor from sludge

Crude sapogenin	Acid liquor (reuse several times)

8. Neutralize with soda ash
9. Dry and grind sludge
10. Extract sludge with boiling hexane or heptane
11. Decant solvent from insolubles

Solution (hecogenin + tigogenin)	Sludge (discard)

12. Treat with decolorizing carbon if necessary
13. Concentrate until crystalization just begins

Insoluble crystals hecogenin	Soluble fraction *tigogenin*

14. Concentrate

Crystal *stigogenin*

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